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Extended Evaluation of an 'In vivo' Teratology Screen Utilizing Postnatal Growth and Viability in the Mouse

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16. ABSTRACT

A teratology test system proposed by Chernoff and Kavlock (3,4) utilizes growth and viability for 3 days after birth to prioritize chemicals for standard teratology testing. The present study is an extended observation of the growth and viability of Chernoff and Kavlock's animals from 41 treatments for 250 days to determine if neonatal weight reductions persisted throughout life, if mortality occurred later in life or, if other serious abnormalities developed that could not be anticipated from the neonatal data. One chemical which was positive in the present study would be a false negative in the standard teratology test and the Chernoff/Kavlock Assay (CKA). Congo red did not cause frank malformations or mortality but it specifically altered reproductive function in the offspring. Two chemicals produced a variety of unusual abnormalities that were not apparent in the neonates, indicating that postnatal testing is necessary to determine the full teratogenic potential of some compounds. Mice exposed prenatally to cytosine arabinoside became hydrocephalic and 30% of them lacked lower incisors. Nitrofen caused hydrocephaly, the age of eye-opening was delayed, a few of the mice had no eyes, and most of the mice lacked Harderian glands. Eighteen of the 41 treatments caused mortality of 15%, or greater, by day 3, and four of these treatments resulted in additional mortality past the neonatal period. No compound caused a decrease in viability after the neonatal period without also reducing days 1 or 3 litter sizes, validating the use of this measurement in the CKA.

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An Extended Evaluation of an In Vivo Teratology Screen Utilizing Postnatal Growth and Viability in the Mouse

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A teratology test system proposed by Chernoff and Kavlock [3,4] utilizes growth and viability for 3 days after birth to prioritize chemicals for standard teratology testing. The present study is an extended observation of the growth and viability of Chernoff and Kavlock's animals from 41 treatments for 250 days to determine if neonatal weight reductions persisted throughout life, if mortality occurred later in life or, if other serious abnormalities developed that could not be anticipated from the neonatal data. One chemical which was positive in the present study would be a false negative in the standard teratology test and the Chernoff/Kavlock Assay (CKA). Congo red did not cause frank malformations or mortality but it specifically altered reproductive function in the offspring. Two chemicals produced a variety of unusual abnormalities that were not apparent in the neonates, indicating that postnatal testing is necessary to determine the full teratogenic potential of some compounds. Mice exposed prenatally to cytosine arabinoside became hydrocephalic and 30% of them lacked lower incisors. Nitrofen caused hydrocephaly, the age of eye-opening was delayed, a few of the mice had no eyes, and most of the mice lacked Harderian glands. Eighteen of the 41 treatments caused mortality of 15%, or greater, by day 3, and four of these treatments resulted in additional mortality past the neonatal period. No compound caused a decrease in viability after the neonatal period without also reducing days 1 or 3 litter sizes, validating the use of this measurement in the CKA. Cytosine arabinoside was administered later in gestation than the CKA dosing regime and caused a marginally significant increase in mortality near birth but killed over 50% of the pups after weaning. Altering the duration or period of exposure in the CKA may necessitate longer postnatal evaluation than presently recommended [3,4]. Neonatal weight, the second parameter used in the CKA to prioritize chemicals for additional testing, appears to be less useful than viability. It was a poor predictor of subsequent growth, some early weight effects persisted, while most of those concurrent with maternal weight reductions were transient. A correlation analysis of the parameters measured in the CKA and in the present study, excluding the

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compounds that produce frank malformations, indicated that compounds that reduced maternal weight gain during dosing had fewer live pups and growth was transiently retarded. Compounds could be tested in the CKA at a dose that results in less than a 20% reduction in maternal weight gain during dosing without confounding maternal toxicity with postnatal effects.

Key words: Teratology, mouse, postnatal evaluations, reproductive effects. developmental toxicity, congo red, azo dye, nitrogen, cytosine arabinoside

INTRODUCTION

Standard protocols have been established for the testing of the teratogenic potential of chemicals [1]. In these experiments pregnant animals, usually rodents, are exposed to chemicals during the period of organogenesis and the fetuses are examined near birth for skeletal and soft tissue abnormalities. Because such testing is expensive to conduct, a number of screening methodologies have been suggested that might be used to quickly and inexpensively prioritize chemicals for standard testing [2]. Recently Chernoff and Kavlock [3,4] proposed an in vivo teratology screen for this purpose. In this assay pregnant mice are exposed to a minimally toxic dose (MTD) of a chemical during the period of major organogenesis, they are allowed to deliver, and the growth and viability of the offspring are observed during the first 3 days after birth. In a study using 40 chemicals of known teratogenic potential, they concluded that this test provided a simple but accurate picture of the response of the mouse embryo in a standard test [4]. Brown et al [5] also presented a favorable evaluation of this test system after examining 55 compounds. It is important to note that the reliability of this screen, like any other test protocol, rests on the validity of certain assumptions which are the focus of the present study. These assumptions include 1) compounds that result in normal survival up to 3 days of age do not induce mortality later in life; 2) the measurement of neonatal growth from birth to 3 days of age is a useful indice of development; and 3) pups with normal survival and growth rates up to 3 days of age do not develop serious anomalies later in life. While no single teratology test system, including the standard test, is perfect and without limitations, it is important to understand the frequency and magnitude with which certain assumptions are violated so that the limitations of such testing can be determined. In an attempt to determine the reliability of these assumptions, the growth, viability, and fertility of the offspring from the CKA test system were observed in the present study through 250 days of age.

In the present study, pregnant mice were exposed to one of the 41 different treatment conditions and the postnatal development of their offspring was observed. Most of the treatment conditions used in this study were selected because their teratogenic or fetotoxic potential has been established in studies using mice. Only after testing many chemicals that have known teratogenic potential in mice were chemicals that have unknown effects utilized in the present study. Many of these unknowns have been tested in rats. This study was designed to determine 1) if the mice that survive prenatal exposure to a teratogen and appear normal continue to develop normally; 2) if growth retardations seen near birth are persistent; 3) if postnatal mortality extends past the early neonatal period; and 4) if chemicals that seem to be nonteratogenic and nonfetotoxic based on the neonatal data induce subsequent alterations of growth, viability, morphology, or fertility.

METHODS

The maternal and neonatal data from most of the compounds used in the present study are presented by Chernoff and Kavlock [3,4]. The present study is an extended observation of their mice. Additional treatments were administered later in gestation than the period utilized by Chernoff and Kavlock to evaluate their hypothesis that the duration of exposure could be altered and the assay would still be valid (Table I).

In the present study, more than 1,500 pregnant mice were exposed to one of 35 different chemicals in 41 different treatment regimens. The dams were allowed to deliver and the postnatal viability, growth, morphology, and reproductive function of the offspring was assessed. The study was conducted over a 2-year period in 21 blocks with two or three treatment groups and a concurrent control per block. Within each block, each treatment group initially contained 23 to 40 primiparous 90-day-old female mice (CD-1) received on day 5 of gestation from Charles River Breeding Laboratories (MA). Dams were housed individually in transparent plastic cages (33 × 20 × 13 cm) with pine shavings as bedding; cages were changed three times per week. The animals were maintained on a 12:12 photoperiod with lights off at 1800 (EST), Wayne Lab Blox and tap water, ad libitum, and room temperature of 20–24°C. Upon arrival dams were randomly assigned to control or treated groups. The treatment groups, doses, and routes of exposure are given in Table II. Unless otherwise noted, the treatments were administered daily on days 8 through 12 of gestation, which is within the period of major embryonic organogenesis. Doses were selected from published studies or from studies in our laboratory that determined a dose that would be minimally toxic to the dam. In studies to determine the MTD, nonpregnant female mice were housed five per cage. Animals were dosed on a mg/kg basis for five consecutive days at one of five dose levels. Each dose level consisted of 10 animals. The MTD was considered that dose which resulted in either significant weight change during the treatment period, mortality, or other signs of toxicity. The dose levels for eight known teratogens (aminopterin, 6-aminonicotinamide, benomyl, 5-bromodeoxyuridine, cortisone, 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], sodium salicylate, and cytosine arabinoside) were taken directly from previous studies in our laboratory or the literature. MTDs could not be determined for thalidomide, sodium salicylate, congo red (oral), and ethylenediaminetetraacetic acid (EDTA). All compounds were at least 97% pure except for congo red (92%), chlordecone (technical grade, approximately 90%), chlordane (72%), alcian blue (55%), toxaphene (68.9% chlorine content), diazo red (50%), and aminopterin (95%).

The females were weighed throughout dosing and on day 19 of gestation. They were allowed to give birth and the litters were counted and weighed at 1 and 3 days of age. Dead pups recovered from the cages were necropsied and abnormalities recorded. Dams that had not given birth by postnatal day 3 were killed and examined for the presence of resorptions.

At this point random samples of the dams and pups were selected from the CKA and utilized for the present study. In the first block, litters were randomly selected for observation in the present study (Table II). In the subsequent blocks, four to 12 dams were randomly selected for each treatment group on postnatal day 6 and given six pups (three males and three females) that were randomly selected from the entire population of pups in that treatment group. Four hundred sixty dams with fostered litters were used; the number of litters in each block is shown in Table III. Sample

TABLE I. Maternal and Perinatal Effects of Prenatal Administration of Test Compounds Not Previously Reported in Chernoff and Kavlock [3,4]

		Treatment regimen				Maternal				Perinatal			
Block	Compound	Dose (mg/kg)	Route	Vehicle	Days	No. treated	No. dead	No. preg.	Weight change (g)	No. live day 1	Avg. weight day 1 (g)	No. live day 3	Avg. weight day 3 (g)
8	Control	0	Oral	Corn oil	14–18	24	2	17	12.7	10.0	1.64	9.8	2.16
	Endrin	2				24	5	12	12.4	10.0	1.67	9.5	2.18
	Chlordecone	20				25	3	15	13.8	10.9	1.62	10.0	1.98
9	Control	0	IP	H_2O	15	25	0	21	23.3[a]	9.3	1.76	9.3	2.38
	6-Aminonicotinamide	10				24	0	14	15.1[b]	8.5	1.32[b]	6.6[b]	1.67[b]
12	Control	0	Oral	Corn oil	8–12	25	0	17	5.9	9.6	1.68	9.6	2.27
	Chlordecone (Repeat)	20				25	1	20	3.8[b]	10.2	1.58[b]	8.3	2.01[b]
14	Control	0	Oral	Corn oil	8–12	25	3	16	7.7	10.8	1.61	10.4	2.08
	Thalidomide	400				25	1	15	7.7	10.8	1.61	10.7	2.16
16	Control	0	Oral	H_2O	8–12	40	1	21	7.3	10.0	1.68	9.8	2.22
	Sodium salicylate	800				30	7	17	6.7	9.5	1.60[b]	9.2	2.00[b]
20	Control	0	IP	Saline	11, 12, 14, 15	40	0	33	—	10.8	1.58	9.5	1.98
	Cytosine arabinoside	30			14, 15	30	0	23	—	9.0	1.41[b]	7.8	1.77[b]

[a]Maternal weight change was calculated from day 19 weight minus day 7 weight, rather than pretreatment minus posttreatment weights as in other blocks.
[b]Mean differed significantly from the control.

sizes for some of the compounds were as small as four or five litters because of excessive treatment-induced mortality. It became apparent in the first block that litter sizes should be reduced to a uniform number in order to reduce the variability in growth resulting from large differences in litter sizes. Rearing pups in large litters is an extreme physiological stress that reduces viability during lactation and permanently retards growth. For example, in one study in our laboratory we found that litters with 12 pups experienced a 55% mortality before weaning as compared to 10% mortality in litters of six pups. In addition, the pups from the large litters were 20% lighter at 120 days of age (Gray, unpublished data). Therefore, pups in litter sizes of six have optimum environmental conditions for growth and viability which enables one to detect alterations in these parameters caused by prenatally induced physiological deficits. It is important to point out that the litters were not culled of sickly pups, which might be the affected populations, but rather the pups were randomly selected. For this reason the effects noted in our sample population should be representative of effects in the entire population. At 22 days of age a male and female from each litter were weighed after behavioral testing as were two males from each litter at 57 days. The behavioral testing consisted of a 1-hr observation of reactive locomotor activity in a novel environment. The behavioral data are not presented in the present report. At 30 days of age the pups were weaned, counted, and weighed and the numbers of females with patent vaginas was determined. The pups remained in bisexual groups after weaning for breeding purposes. Throughout the remainder of the experiment the animals were checked while the cages were being changed and obviously pregnant females were removed and individually housed until parturition when litter size and age of the dam were recorded. No other measurements were made on the females and they were not returned to their home cages after the first litter. Ovarian and uterine weights were not taken in the present experiment because these values cycle along with the estrous cycle and parity.

At approximately 250 days of age the males were necropsied and body weight and weights of the liver, testes, seminal vesicles, and right kidney were recorded. The organs weighed at this time only reflect an interest in reproductive and kidney physiology and were not part of a comprehensive gross pathological analysis. However, any obvious gross pathology was recorded. While many systems were not examined at all in our postnatal evaluation these systems have not been entirely ignored because they are essential for normal growth and viability. For example, it is likely that severe alterations of endocrine or organ function would retard growth and reduce postnatal viability.

The postweaning protocol of blocks 20 and 21 was modified to improve our test. At 35 days of age a single male was housed with a single nonlittermate female instead of housing three males and three females together. This was done because one fertile male in the group could fertilize all the females and the survival of males after breeding was poor owing to occasionally high levels of intermale aggressive interactions. In addition, in these blocks the males were necropsied at 150 days of age.

In the first 19 blocks, survival rates after weaning were calculated from the numbers of females alive through day 60 divided by the numbers of females weaned. In blocks 20 and 21, where there were only one male and female per cage, the data from both sexes was used to calculate postweaning survival rates.

The body weight data on day 30 was collected and analyzed by univariate ANOVA using the litter means. All other data were analyzed using individual values

TABLE II. The Effects of Prenatal Exposure to Chemicals on the Postnatal Growth and Organ Weights of the CD-1 Mouse*

Chemical	Block	Dose (mg/kg/day)	Route	Day 3 No. Live (M + F)	Day 3 body weight (M + F)	Day 22 body weight (M + F)	Day 30 body weight		Day 57 body weight	Weights at necropsy				
							M	F	(M)	Body (M)	Liver (M)	Testes (M)	Seminal vesicle (M)	Kidney (M)
Control	1	0	Oral	9.1	1.98	8.3	22.1	19.3	30.2	43.2	2.485	226	550	369
Dinoseb		15		9.9	2.00	8.5	22.7	20.3	29.9	42.1	2.264	269	543	382
Carbaryl		100		9.5	2.15	10.1	24.5	21.5	32.4	45.5	2.489	253	556	379
Control	2	0	Oral	9.3	2.33	12.9	26.3	22.0	32.7	54.7	2.998	282	629	420
Toxaphene		75		9.3	2.11	11.3	25.1	21.0	32.2	50.7[a]	2.856	278	548[a]	450
Vitamin A (IU/kg)		160,000		3.8[a]	2.26	11.6	25.1	21.4	32.6	44.8[a]	2.473	320	501[a]	398
Control	3	0	Oral	10.2	2.35	12.5	22.2	19.7	32.7	47.4	2.740	281	556	430
Endrin		2		9.3	2.26	12.3	22.6	19.8	33.0	48.9	2.817	288	481	415
Chlordecone		20		7.3[a]	2.00[a]	11.3	21.9	18.7	31.7	50.0	2.698	273	486	386[a]
Control	4	0	Oral	9.3	2.25	13.6	28.5	23.2	34.7	52.1	3.088	309	597	438
Caffeine		200		7.6	2.05[a]	11.6[a]	26.7	22.4	33.8	49.5	2.866	299	546	398
Cacodylic acid		600		7.1	1.89[a]	12.5	26.7	22.7	31.5[a]	45.7[a]	2.442[a]	252[a]	449[a]	387[a]
Control (day 8)	5	0	IP	9.7	2.55	12.7	28.0	23.1	34.0	50.3	2.531	293	537	386
5-Bromodeoxyuridine (day 8)		400		4.9[a]	2.43	13.0	27.1	22.6	34.5	54.4	2.576	302	547	385
Control	6	0	Oral	10.6	1.89	12.0	25.2	20.9	34.2	50.8	2.677	264	582	410
Benomyl		200		3.9[a]	1.81	10.4	21.1[a]	16.7[a]	31.3[a]	49.2	2.729	224[a]	483	394
Control	7	0	Oral	10.4	2.17	14.2	26.8	22.6	34.5	52.5	2.618	289	495	410
Ethylenethiourea (ETU)		300		10.0	2.31	14.2	26.8	22.6	35.0	51.1	2.730	302	554	425
Ethylenediaminetetraacetic acid (EDTA)		1,000		9.0	2.28	14.6	28.0	22.7	36.3	52.1	2.765	288	529	405

TABLE II –continued.

Control	8	0	Oral	9.8	2.16	14.6	29.6	23.6	36.6	54.2	3.090	287	567	423
Endrin (days 14–18)		2		9.5	2.18	13.0	29.1	24.4	35.8	58.7	3.190	298	722	456
Chlordecone (days 14–18)		20		10.0	1.98	13.6	29.2	25.3	34.2	51.6	2.830	279	494	402
Control (days 11, 15)	9	0	IP	9.3	2.38	14.0	29.7	24.9	37.0	52.5	3.190	305	537	458
6-Aminonicotinamide (day 11)		10		4.4[a]	1.90[a]	12.5[a]	26.3	22.6	29.2[a]	46.0[a]	2.900	246[a]	460	373[a]
6-Aminonicotinamide (day 15)		10		6.6[a]	1.67[a]	12.2[a]	26.8	23.0	34.2	47.4	2.930	280	521	407
Control	10	0	Oral	9.5	2.44	14.1	26.7	23.4	34.8	52.2	3.270	286	665	432
Decamethrin		10		11.4	2.27	14.3	26.8	23.1	35.1	51.7	3.170	282	505	438
Ethylenebisisothiocyanate (EBIS)		200		11.3	2.22	14.2	26.9	23.9	34.7	56.8	3.730	308	542	462
Control	11	0	Oral	8.9	2.43	15.9	28.3	24.1	34.9	57.7	3.790	297	586	478
Hexachlorocyclopentadiene		45		9.9	2.36	15.3	27.2	23.4	34.5	55.8	3.780	286	501	444
Cyclohexanone		800		10.7	2.34	14.7	28.0	23.1	35.9	55.3	3.620	305	562	480
Control	12	0	Oral	9.6	2.27	13.7	27.5	23.6	35.1	52.9	3.340	279	569	466
Chlordecone		20		8.3	2.01[a]	13.7	26.8	22.9	33.3	51.8	3.090	280	671	424[a]
1,2,4 Trichlorobenzene		130		9.6	2.35	13.2	26.9	23.9	35.2	53.6	3.010	288	550	453
Control	13	0	Oral	10.7	2.21	14.7	28.0	23.8	35.8	55.8	3.480	283	585	475
Chlordane		50		9.9	2.19	14.0	27.4	23.3	36.6	57.3	3.970	299	712	520
Lindane		25		9.7	2.38	13.9	26.7	18.7	35.9	56.2	3.450	294	582	435
Control	14	0	Oral	10.4	2.08	13.9	25.9	22.7	33.7	52.0	3.230	294	592	478
Mirex		7.5		4.1[a]	1.73[a]	13.1	24.3	20.5	33.1	50.4	3.260	274	503	394[a]
Thalidomide		400		10.7	2.16	13.1	24.3	20.4	33.5	50.6	3.360	282	567	424
Control	15	0	Oral	10.8	2.08	11.5	24.8	24.4	32.5	50.6	3.180	283	470	420
2,4,5 Trichlorophenoxy acetic acid		130		7.1[a]	2.14	12.5	25.2	21.8	34.5	55.3	3.560	295	522	453
Nitrofen		200		1.9[a]	1.55[a]	9.9[a]	21.8	19.6	32.8	44.3[a]	—	—	—	—
Control	16	0	Oral	9.8	2.22	11.3	25.7	21.4	34.5	50.9	3.330	292	456	431
Sodium salicylate		800		9.2	2.00*	11.7	25.2	21.4	35.1	52.8	3.500	300	542	417
Congo red		1,000		9.3	2.01*	12.2	24.9	22.4	32.7	47.9	2.980	210[a]	406	403

TABLE II-Continued.

Chemical	Block	Dose (mg/kg/day)	Route	Day 3 No. Live (M + F)	Day 3 body weight (M + F)	Day 22 body weight (M + F)	Day 30 body weight M	Day 30 body weight F	Day 57 body weight (M)	Weights at necropsy: Body (M)	Liver (M)	Testes (M)	Seminal vesicle (M)	Kidney (M)
Control	17	0	Oral	9.6	2.07	12.8	26.2	21.1	33.5	48.9	3.078	275	480	432
Kelevan		125		9.5	1.97	11.6	24.1	21.1	32.1	45.9	2.878	291	471	443
Nickel chloride		100		10.7	2.07	11.2	25.0	20.2	34.1	48.7	2.752	279	494	434
Control (day 8)	18	0	IP	9.1	2.13	13.0	22.0	19.7	34.0	50.1	3.141	265	506	401
Nickel chloride (day 8)		30		6.2	2.11	13.2	21.8	19.3	32.6	46.1	2.785	282	504	415
Diazo red RD (day 8)		150		9.3	1.80[a]	12.0	20.8	18.7	33.9	50.2	2.916	285	506	383
Alcian blue (day 8)		250		3.5[a]	1.95	11.0	18.4[a]	17.0[a]	32.9	45.8[a]	2.610[a]	272	436	424
Control (day 8)	19	0	IP	10.6	2.13	15.4	26.0	21.6	35.3	48.8	2.855	309	464	415
Congo red (day 8)		300		7.4[a]	2.00	13.0[a]	24.9	21.6	35.0	50.5	3.012	244[a]	568	451
Control (days 11, 12, 14, 15)	20	0	IP	9.5	1.98	14.9	26.1	21.0	—	44.0	2.921	265	368	405
Aminopterin (day 11)		20	IP	0.8[a]	2.15	12.8	24.9	19.1	—	39.8[a]	2.317[a]	276	288	378
Cortisone (day 12)		300	IM	1.3[a]	1.63[a]	11.6[a]	20.3[a]	18.7[a]	—	35.0[a]	2.199[a]	229	323	320
Cytosine arabinoside (days 14/15)		30	IP	7.8	1.77[a]	9.0[a]	15.6[a]	11.7[a]	—	34.6[a]	2.305[a]	275	345	449
Control	21	0	Oral	11.1	2.15	14.4	26.8	22.6	—	43.1	2.614	281	350	358
Thalidomide		2,000		10.3	2.21	13.7	27.6	23.4	—	44.3	2.668	277	338	372
2,4,5 Trichlorophenol		800		9.8	2.12	14.6	26.1	23.1	—	42.9	2.648	271	341	371
Sodium selenite		10		9.4[a]	2.17	14.2	27.4	22.8	—	43.6	2.746	274	368	366

*Dams were dosed on days 8–12 of gestation unless otherwise noted. The males were necropsied at day 250 in blocks 1 through 19 and at 150 days in 20 and 21.

[a]Mean differed significantly from control.

rather than litter means because, with the exception of block 1, the pups within each litter were not genetic littermates. The necropsy data were analyzed by block using a multivariate ANOVA. The data on age at parturition and F1 litter sizes were also analyzed by block using a multivariate analysis of variance. When the MANOVA indicated the presence of a significant treatment effect the data were partitioned and reanalyzed using a univariate ANOVA. The percentage data shown in Table III were analyzed by computing a normal deviate z, derived from the normal approximation to the binomial. The z and the chi-square tests are identical. In addition, the control data were analyzed to determine if any of the variables exhibited significant block effects. Correlation matrices were calculated for 1) the control groups, 2) the compounds that are known to cause frank malformations or resorptions, and 3) the compounds that do not cause malformations or resorptions using group means of the maternal and neonatal data from the CKA study and data from the present study.

RESULTS

General

The effects of prenatal administration of most of the chemicals on neonatal viability and weight and maternal viability and weight change during dosing are presented in Chernoff and Kavlock [3,4]. New data on these parameters for treatments not presented earlier are shown in Table I. The effects on subsequent postnatal growth and organ weights at necropsy are presented in Table II. Effects on viability, reproductive function, and pathology are in Table III. The viability and body weights of the offspring on day 3 (from Chernoff and Kavlock [3,4]) are presented in Table II for comparative purposes with the data collected in the present study from a random sample of the same mice. A summary of all effects noted in both the CKA and the present study are contrasted with the results reported in the literature for each treatment in Figure 1.

The analysis of the control data indicated that there were significant block effects for all parameters measured in the present experiment including growth, viability, and reproductive performance. This indicates that treatments could be compared only with the concurrent control group. Similar block effects were observed by Chernoff and Kavlock [3,4] on maternal weight, maternal weight gain, litter sizes, and pup weight on days 1 and 3 in the first phase of this experiment; Butcher [6] reported large interlaboratory differences between five laboratories using uniform test procedures, and Schwetz and Rao [7] reported a range in their Fertility Index from 44 to 100% in the control groups over eight reproduction studies over a 9-year period in their lab. These block effects do not statistically confound the interpretation of the data in any of these studies but they do suggest that subtle alterations seen in the CKA and the present can differ significantly from the concurrent control group but also fall within the normal range of the control values from all the blocks. We would recommend that subtle effects be replicated before doing more extensive testing and that guidelines be utilized to assure that each control group is within the normal range. If a control deviates from normality the entire block should be replicated.

Maternal Effects

Six treatments caused maternal death but the surviving dams gained weight normally during gestation. Four treatments caused maternal death and reduced maternal weight gain, and ten compounds were administered at a dose that reduced maternal

TABLE III. The Effects of Prenatal Exposure to Chemicals on the Reproductive Development and Morphology of the CD-1 Mouse*

Chemical	Block	No. of litters	Percent viability to day 30	Percent vaginal patency day 30	Percent pregnant	Age at parturition	F1 litter size	Abnormalities
Control	1	4	—	24				
Dinoseb		6	—	57				
Carbaryl		3	—	87				
Control	2	6	92	86	100	65.2	10.7	
Toxaphene		6	100	29[a]	93	64.4	8.2	
Vitamin A (IU/kg)		4	88	64	90	64.9	9.8	EN, HM, KT (1)
Control	3	8	98	70	95	68.4	10.9	
Endrin		6	98	71	88	64.7	9.9	
Chlordecone		6	100	63	96	68.0	9.1	
Control	4	8	85	100	94	65.7	10.9	
Caffeine		8	96	100	90	66.1	9.5	
Cacodylic Acid		6	90	91	83	63.4	8.7	
Control (day 8)	5	8	92	71	62	64.3	11.2	
5-Bromodeoxyuridine (day 8)		8	83	67	94	64.6	11.6	EN
Control	6	8	88	84	78	61.7	9.7	
Benomyl		8	65[a]	50[a]	67	64.7	9.9	EN, HM, KT, (many)
Control	7	8	93	100	73	62.6	12.3	CT (1)
Ethylenethiourea (ETU)		8	100	95	85	62.6	11.3	
Ethylenediaminetetraacetic acid (EDTA)		8	94	100	82	63.2	12.1	
Control	8	8	73	100	85	64.2	10.6	
Endrin (days 14–18)		8	94	100	71	63.9	9.7	
Chlordecone (days 14–18)		8	56[a]	92	79	66.4	9.9	CT (1)
Control (days 11, 15)	9	8	89	70	100	67.8	11.0	MAL (1)

TABLE III–continued.

6-Aminonicotinamide (day 11)		8	94	25[b]	67[b]	62.6[b]	9.9	KT (70%)
6-Aminonicotinamide (day 15)		8	87	64	95	66.3	11.2	
Control	10	8	94	100	86	64.0	12.6	
Decamethrin		8	90	94	88	64.2	11.5	
Ethylenebisisothiocyanate (EBIS)		8	83	100	76	65.6	13.1	
Control	11	8	97	100	100	66.3	13.0	
Hexachlorocyclopentadiene		8	94	100	89	64.4	13.0	KT (1)
Cyclohexanone		8	96	88	95	65.2	11.7	HT (5)
Control	12	8	90	90	79	66.3	11.1	
Chlordecone		8	89	95	100	64.5	11.4	
1,2,4 Trichlorobenzene		8	93	91	100	67.0	12.1	
Control	13	8	96	90	89	64.3	13.3	
Chlordane		8	89	100	100	65.0	11.8	
Lindane		8	88	92	84	63.7	10.2[b]	
Control	14	7	69	66	87	65.7	11.4	
Mirex		6	80	66	83	63.6	10.1	DA
Thalidomide		8	70	81	78	63.9	10.9	
Control	15	8	100	83	96	65.6	10.7	
2,4,5 Trichlorophenoxy acetic acid (2,4,5-T)		8	100	95	100	65.0	12.3	CP, TA (1)
Nitrofen		4	79[a]	91	100	64.5	11.2	CP, DA, ES, HC
Control	16	12	86	90	100	67.7	11 4	
Sodium salicylate		8	95	90	100	67.3	11.4	KT (1)
Lithium carbonate		8	88	89	100	66.6	10.2	TA (1)
Congo red (oral)		8	100	85	90	70.6[a]	9.5[a]	TA (25%)
Control	17	10	91	90	90	66.0	10.9	
Kelevan		8	98	86	86	66.2	10.1	KT (1)
Nickel chloride		8	81	71	94	65.9	9.7	KID (1)

TABLE III - Continued.

Chemical	Block	No. of litters	Percent viability to day 30	Percent vaginal patency day 30	Percent pregnant	Age at parturition	F1 litter size	Abnormalities
Control (day 8)	18	8	100	29	100	63.1	10.8	
Nickel chloride (day 8)		5	90	18	91	65.8	10.5	SS, MAL, MO (1)
Diazo red RC (day 8)		8	100	10	90	63.6	11.2	
Alcian blue (day 8)		4	100	8	92	66.1	11.2	T (1)
Control (day 8)	19	4	100	75	100	65.2	10.9	
Congo red (day 8)		6	100	69	100	64.8	9.6	
Dimethyl sulfoxide (20 mcl, SC, PN D2)		3	63	13	100	69.4	11.1	
Chlordecone (500 mcg, SC, PN D2)		3	94	15	8[b]	68.0	11.0	
Estradiol benzoate (50 mcg, SC, PN D2)		3	90	0	8[b]	65.0	4.0	
Control (days 11, 12, 14, 15)	20	10	68	47	88	68.5	12.7	
Aminopterin (day 11)		3	100	29	100	70.9	11.3	
Cortisone (day 12)		5	80	20	100	67.1	9.9[c]	CP (many)
Cytosine arabinoside (days 14, 15)		12	80	4[a]	64	71.8	10.4	CP (4), HC (30%), MO (3), LI (30%)
Control	21	10	100	55	94	64.7	13.9	
Thalidomide		8	98	39	100	64.4	13.6	
2,4,5 Trichlorophenol		8	94	94	93	66.3	11.4[c]	
Sodium selenite		8	100	55	93	65.4	12.4	

*Dams were dosed on days 8–12 of gestation unless otherwise noted. Abnormalities noted throughout the experiment included encephalocele (EN), hemimelia (HM), kinky tail (KT), hematoma on tail (HT), cryptorchid testis (CT), malocclusion (MAL), distended abdomen (DA), cleft palate (CP), eyes shut after weaning (ES), hydrocephaly (HC), testicular atrophy (TA), kidney absent (KID), short snout (SS), tailless (T), microphthalmia (MO), and lower incisors missing (LI). The values in parentheses under Abnormalities indicate the number or percentages of offspring affected.

[a]$P < .05$ different from control.

[b]$P < .01$.

[c]$P < .05$ using a one-tailed t test.

Legend: ˙ Slight effect + Increased – Decreased M Teratogenic malformations D Fetal death W Fetal weight N No effect U Unknown effects EX Extra ribs		RESULTS REPORTED BY CHERNOFF AND KAVLOCK (4)								DEVELOPMENTAL ALTERATIONS SEEN IN THE PRESENT STUDY													
COMPOUND	RESULTS OF TERATOLOGY STUDY	Maternal Death	Maternal Weight	Whole Litters Resorbed	Malformations in Pups	Day 1 Live	Day 3 Live	Day 1 Weight	Day 3 Weight	Malformations not seen in (4)	Viability: Day 3-30	Viability: Day 30-60	Body Weight: Day 22	Body Weight: Day 30	Body Weight: Day 67	Body Weight: Day 250	Liver Weight	Kidney Weight	Testes Weight	Seminal Vesicle Weight	Vaginal Opening (%)	Age at Parturition	(%) Pregnant	F_1 Litter Size
Aminopterin	M		–	+		–	–									–	–							
6-Aminonicotinamide D11	M		–		+	–	–	–	–				–		–	–		–			–	–	–	
6-Aminonicotinamide D15	M		–			–	–	–	–				–											
Benomyl	M				+	–	–	–			–			–	–				–			–		
5-Bromodeoxyuridine	M				+	–	–																	
Cacodylic Acid	M	+	–	+		+	+	–	–						–	–	–	–	–	–				
Cortisone	M		–	+	+	–	–	–	–				–	–		–	–							
Cytosine Arabinoside	M				+	–	–	–	–	+		–	–	–		–	–					–		
Mirex	M				+	–	–	–	–									–						
Nickle Chloride (ip)	M		–	+		–	+																	
Nitrofen	M				+	–	–	–	–	+	–		–	–		–								
2,4,5 - T	M			+		–																		
Vitamin A	M		–		+	–	–	–								–				–				
Chlordecone	D	+	–			–	–	–	–									–						
Endrin	W		–					–																
Sodium Salicylate	W							–	–															
Sodium Selenite	W		–			–	–																	
Decamethrin	EX																							
ETU	EX	+																						
Caffeine	N		–				+		–				–											
Carbaryl	N																							
Chlordane	N	+																						
Dinoseb	N																							
EPIS	N	+																						
Hexachlorocyclopentadiene	N																							
Toxaphene	N		–					–								–				–	–			
Thalidomide (2 doses)	N																							
2,4,5 Trichlorophenol	N	+			–																			
Alcian Blue	U	+	–	+		–	–							–		–	–							
Chlordecone (d14-18)	U																							
Congo red (ip)	U	+	–	+		–	–			+			–						–					
Congo red (oral)	U								–	+									–			•		–
Cyclohexanone	U																							
Diazo red RC	U							–	–															
EDTA	U																							
Endrin (14-18)	U	+																						
Kelevan	U	+																						
Lindane	U																							–
Nickle Chloride (oral)	U																							
1,2,4 Trichlorobenzene	U																							

Fig. 1. A summary of the effects of prenatal exposure to chemicals used in the present study. The results from a standard teratology bioassay examining fetal mice have been described by Chernoff and Kavlock [4]. The results of their in vivo screen are also contrasted with the developmental alterations seen from days 4 to 250 in the present study.

TABLE IV. Correlation Matrices for Maternal and Neonatal Parameters*

	Maternal weight change during treatment (MWT)	No. live pups day 1	No. live pups day 3	Pup weight day 1	Pup weight day 3	Maternal mortality (%)	
MWT	—	−.21 NS	−.11 NS	.31 NS	.30 NS	−.04 NS	Control correlations
No. live day 1	.61 P < .004	—	.91 P < .0001	−.75 P < .0003	−.66 P < .003	.35 NS	
No. live day 3	.83 P < .0001	.86 P < .0001	—	−.61 P < .007	−.55 P < .02	.47 P < .05	
Pup wt. day 1	.68 P < .0009	.18 NS	.47 P < .02	—	.87 P < .0001	−.22 NS	
Pup wt. day 3	.34 NS	.19 NS	.45 P < .03	.75 P < .0001	—	−.15 NS	
Maternal mortality (%)	.10 NS	−.33 NS	−.19 NS	.11 NS	−.34 NS		

*Correlations for the compounds that do not produce abnormalities, ie, known negatives, fetotoxins that reduce fetal weight or that cause fetal mortality, and unknowns that did not cause abnormalities in the pups.

weight gain without causing mortality. Of the six treatments that caused maternal death alone only 2,4,5 trichlorophenol had an effect on any parameter measured in the CKA or the present study reducing the number of live pups on day 1. However, all 14 of the compounds that reduced maternal weight gain during treatment were associated with postnatal developmental alterations. Further analysis of the data indicated that a highly significant correlation between maternal weight gain during treatment from days 8–12 and both the number of live neonates and pup weight when compounds that caused frank malformations of resorptions were deleted (Table IV). In the control groups maternal weight was unrelated to any of the neonatal parameters but larger litters tended to weigh less. An inspection of Figures 2 and 3 demonstrates that the apparent relationship between maternal weight and neonatal viability and pup weight is heavily influenced by chlordecone, caffeine, endrin, and toxaphene, treatments that caused greater than 30% reductions in maternal weight gain. Compounds with less of an effect on the dam are distributed normally. These results do not prove that a reduction in maternal weight during treatment caused pup mortality or reduces pup weight, but that an association between these parameters exists in the present data set. These results do suggest that other investigators using the CKA should inspect their data for such relationships. It is clear that compounds that caused maternal death without affecting maternal weight and those that reduced maternal weight by less than 20% did not cause developmental alterations in the present study.

Viability

Eighteen of the 41 prenatal treatment conditions reduced the numbers of live pups on day 3 by 15% or more, indicating that they were fetotoxic or teratogenic (Table V). The 24% reduction in live pups on day 3 owing to cacodylic acid exposure was not significant because seven of 24 dams died, reducing the sample size. The 18% reductions in day 3 viability owing to caffeine exposure was also not significant. Of the 18 chemicals that reduced litter size on day 3 by 15% or more, only two (benomyl and nitrofen) caused additional mortality between 3 and 30 days of age. Cytosine arabinoside drastically increased mortality after weaning. This compound caused an 18% reduction in viability up to day 3 and did not increase mortality between 3 and 30 days of age but produced a 53% decrease in viability after weaning. The neonatal mortality rate of 18% was significant using a one-tailed test but not with a two-tailed analysis even though there were 33 control and 24 cytosine arabinoside litters (Table I).

The postnatal viability of the pups in those 24 treatments that did not alter the numbers of live pups to day 3 was normal.

Gross Abnormalities That Became Apparent After the Neonatal Period

Prenatal administration of nitrofen or cytosine arabinoside caused serious abnormalities that could not be detected in neonates but were eventually incompatible with life. Some of the nitrofen-treated pups became hydrocephalic and died before weaning, eye-opening was delayed as much as 2 weeks, and most animals lacked Harderian glands (Fig. 4), and many of the cytosine arabinoside pups did not have lower incisors (Fig. 5) or became hydrocephalic and died.

Growth

Seventeen of the 41 prenatal treatments caused a growth retardation greater than 8% at some point in the study (Table VI), a level that usually achieved statistical

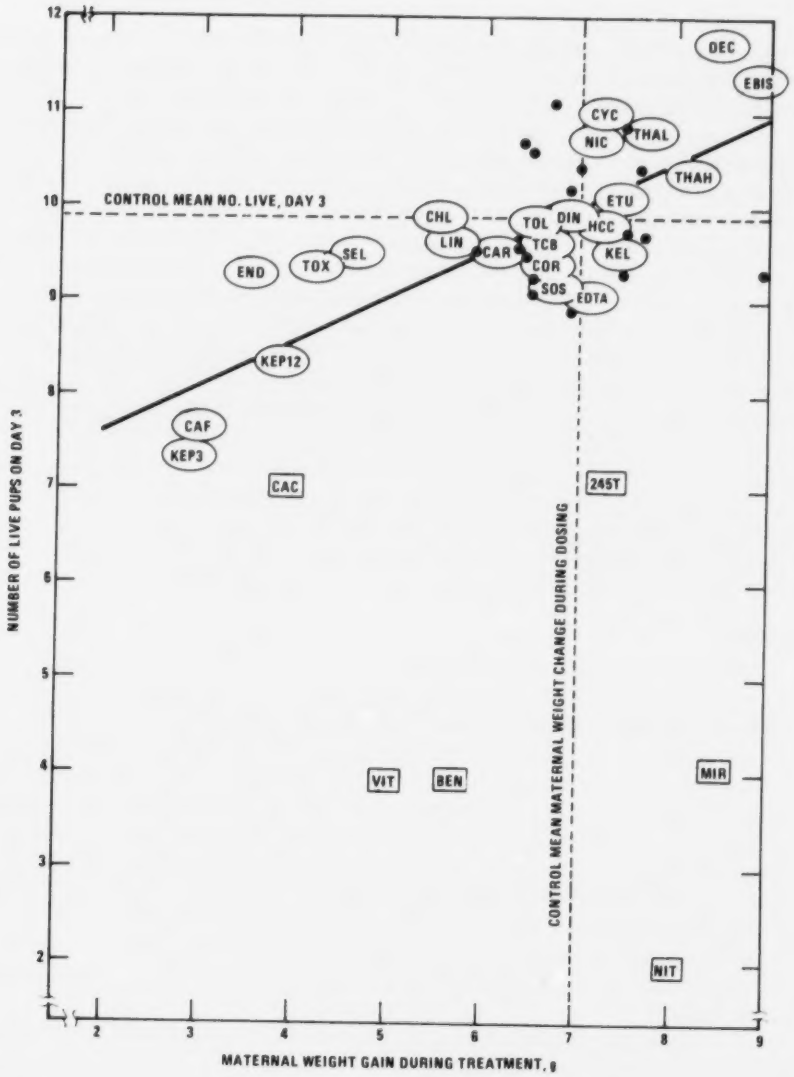

Fig. 2. Compounds that were used in the calculation of the significant correlation ($r = .82$; $P < .0001$) between maternal weight change during treatment on days 8 to 12 of gestation and the number of live pups on postnatal day 3 include fetoxic chemicals that do not cause malformations (KEP 3 = chlordecone [kepone] in block 3; KEP 12 = kepone in block 12; DEC = decamethrin; END = endrin; SEL = sodium selenite; ETU = ethylene thiourea; SOS = sodium salicylate), known negatives (CAF = caffeine; CAR = carbaryl; CHL = chlordane; DIN = dinoseb; EBIS = ethylenebisisothiocyanate; HCC = hexachlorocyclopentadiene; TOX = toxaphene; THAL = thalidomide [400 mg/kg/day]; THAH = thalidomide [2,000 mg/kg/day]; TOL = 2,4,5 trichlorophenol), and unknowns that did not produce malformations or resorptions (COR = congo red, oral; CYC = cyclohexanone; EDTA = ethylenediaminetetraacetic acid; KEL = kelevan; LIN = lindane; NIC = nickle chloride, oral; TCB = trichlorobenzene). The values obtained for compounds that are known to cause malformations, which were not used to calculate the above correlation, are shown in boxes rather than circles and include BEN = benomyl; CAC = cacodylic acid; MIR = mirex; NIT = nitrofen; 2,4,5T = 2,4,5 trichlorophenoxyacetic acid; VIT = vitamin A. The control values are shown as ● on the graph. Number of live on day 3 = 6.662 + (.4759) maternal weight change; $R^2 = 68\%$.

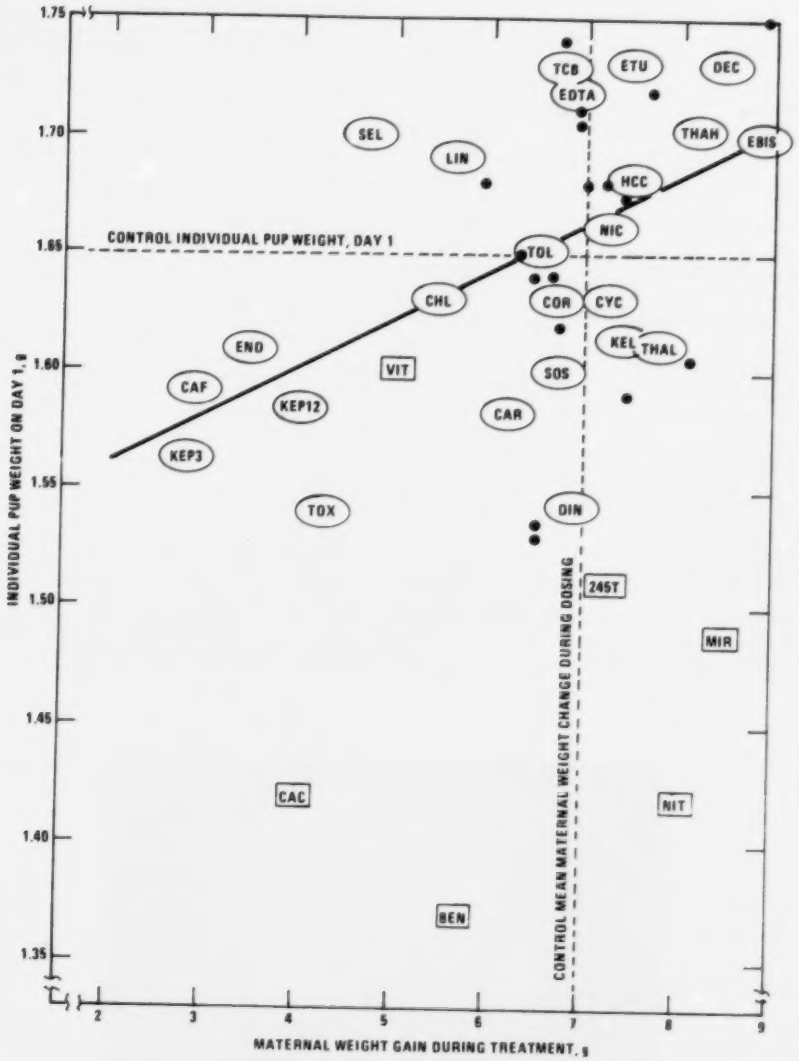

Fig. 3. Correlation of maternal weight change during treatment on days 8–12 of gestation with pup weight on day 1 after birth. Details are the same as Figure 2. Compounds that do not cause malformations appear to reduce pup weight on day 1 ($r = .68$; $P < .0009$) when administered at doses that reduce maternal weight gain during treatment. Pup weight on day 1 = 1.521 × (.01962) maternal weight change; $R^2 = 30\%$.

TABLE V. The Effects of Prenatal Exposure to Chemicals on the Postnatal Viability of the Offspring*

Treatment	Relative mortality due to treatment		
	Day 3	Days 3 to 30	Days 30 to 60
Aminopterin	92%	−31	0
Cortisone	86	−12	12
Nitrofen	82	21	−4
Benomyl	63	23	15
Alcian blue	62	0	0
Mirex	56	−11	0
6 Aminonicotinamide (day 11)	53	−5	−5
Bromodeoxyuridine	51	9	0
Vitamin A	41	10	17
2,4,5 Trichlorophenoxy acetic acid	34	0	−4
Nickel chloride (IP)	32	10	0
Congo red (IP)	30	0	0
6 Aminonicotinamide (day 15)	29	2	15
Cacodylic acid	24	−5	−14
Chlordecone (days 8–12) (blocks 3 and 12)	21	−2	7
Cytosine arabinoside	18	−12	53
Caffeine	18	4	0
Sodium selenite	15	0	0

*The data presented include those chemicals which reduced viability by more than 15% on day 3. The data demonstrate the lack of correlation between day 3 survivorship and subsequent viability. Relative mortality = percent survival of the concurrent control − percent survival in the treated group.

Fig. 4. The gestational administration of the herbicide nitrofen at 200 mg/kg/day on days 8–12 destroys the Harderian gland in the orbit resulting in orbital defects like those seen here while the eyes of the newborn pups appear normal.

Fig. 5. The IP administration of 30 mg/kg/day of cytosine arabinoside on days 14 and 15 of gestation caused the death of 53% of the offspring after weaning. Thirty percent of the pups lacked lower incisors, like the mouse in this figure.

significance. There was a striking lack of correlation between reductions in body weight on days 1 or 3 (Table II) and subsequent growth. Of the compounds that caused significant reductions in neonatal weight, half of these effects were transient and half persisted. In the case of cytosine arabinoside, the effects not only persisted but also became more severe (Table VI), the 11% reduction on day 3 reached 40% on day 22. Diazo red produced a 15% reduction in day 3 weight but subsequent growth was normal, and similar effects were noted with chlordecone (days 8–12), endrin (days 8–12, 6% reduction on day 1 not shown in Table VI), caffeine (9% reduction), mirex (17%), and sodium salicylate (10% reduction). Of the compounds that caused transient effects, endrin, caffeine, and chlordecone had also reduced maternal weight gain during treatment by more than 30%. The weight reduction noted in the 6-aminonicotinamide (day 15) offspring on day 3 (30%, the greatest loss caused by any of the chemicals) persisted but was attenuated to 13% by day 22. The neonatal growth retardations caused by cortisone, cacodylic acid, nitrofen, toxaphene, and 6-aminonicotinamide (day 11) persisted. All of these compounds are known teratogens except for toxaphene, which was administered at a maternally toxic dose.

Four chemicals (alcian blue, benomyl, vitamin A, and aminopterin) reduced growth even though days 1 and 3 body weights were normal. All of these reduced neonatal viability at day 3.

Body, Liver, Seminal Vesicle, and Kidney Weights of Male Mice at Necropsy

The MANOVAs and subsequent ANOVAs indicated that 10 of the 41 treatment conditions caused permanent reductions in body weight (Table II). These treatments included chemicals that did not reduce neonatal weight (vitamin A, alcian blue, aminopterin, and benomyl) and those that did (cytosine arabinoside, cortisone, nitrofen, 6-aminonicotinamide [day 11], toxaphene, and cacodylic aicd). Of the treatments that reduced body weight, five also reduced liver weight, three reduced seminal vesicle weight, and two lowered kidney weight at necropsy. Effects on organ weights

TABLE VI. The Effects of Prenatal Exposure on the Postnatal Growth of the Offspring*

Treatment	Relative body weight		
	Day 3	Day 30	Day 250
6 Aminonicotinamide (day 15)	−30%	−9	−10
Nitrofen	−24	−16	−12
6 Aminonicotinamide (day 11)	−20	−10	−12
Cortisone	−18	−16	−20
Mirex	−17	−8	−3
Cacodylic acid	−16	−4	−12
Diazo red RC	−15	−5	0
Chlordecone (days 8–12)	−13	−3	+2
Cytosine arabinoside	−11	−42	−21
Sodium salicylate	−10	−1	+4
Caffeine	−9	−5	−5
Congo red (oral)	−9	−3	−7
Toxaphene	−9	−5	−7
Alcian Blue	−8	−15	−9
Benomyl	−4	−18	−3
Vitamin A	−3	−4	−12
Aminopterin	8	−6	−9

*The data are for those chemicals which retarded growth at some point in the experiment. The data are presented as the weight of the treated group minus the weight of the concurrent control group divided by the concurrent control group times 100. A correlation analysis of the data indicated that day 3 weight was not correlated with weight at any of the subsequent ages.

in the absence of body weight effects were seen in two cases. Chlordecone (days 8–12 of gestation) in both blocks 3 and 12 and Mirex in block 14 reduced kidney weight without affecting body weight. It is important to note that the effect of chlordecone (days 8–12 of gestation) on kidney weight at necropsy was replicable, even though the blocks differed significantly in necropsy weights.

Reproductive Effects

The injection of estradiol benzoate or chlordecone on day 2 after birth as positive controls drastically reduced the fertility rate of females (Table III, block 19). The administration of 6-aminonicotinamide on day 11 of gestation reduced fertility by 34% and appeared to accelerate the parturition age. Possibly, females that were going to breed were quickly inseminated by the males and those that would have been bred later did not cycle at all. Body and reproductive organ weights of males in this treatment group were lower than controls, but not sufficiently to suggest that the males were sterile.

Oral administration of congo red on days 8–12 of gestation caused a 30% reduction in testes weights, delayed breeding until 50 days of age (the mean age at breeding in the controls in the present study is the remarkedly young age of 45.4 days of age), and reduced F1 litter size by two pups ($P = .003$, MANOVA). The use of mean testes weight for this group is somewhat misleading as it was clear that there were two distinct populations of treated males: most of the treated males had normal testes weighing about 290 mg while the other 25% had testes weighing less than 100 mg. The administration of congo red (IP) on day 8 of gestation was less effective than repeated oral dosing, but it also reduced testes weight. The oral administration of this

chemical was not teratogenic or fetotoxic as indicated by the number of live pups on days 1 or 3, while the injection of congo red resulted in resorptions of whole litters in 4 of the 15 pregnant females. The effect of the oral treatment with congo red on body weight at day 3 seen in the present study and reported by Chernoff and Kavlock [4] did not replicate in a subsequent study, although the testicular effect did [8].

Other reproductive effects seen in the present study include significant reductions in testes weight owing to the prenatal exposure to benomyl, reduced F1 litter size as a result of prenatal lindane treatment ($P = .002$), and a number of compounds reduced the percentage of females with patent vaginas at 30 days. Similar prenatally induced effects of benomyl on testes weights have been reported in studies using rats [9]. Cortisone and 2,4,5 trichlorophenol also lowered F1 litter size (as indicated by a t-test), but these effects were not significant by MANOVA or ANOVA. The three effects on the F1 litter size noted here should be replicated as no other reproductive effects were noted and all three were statistically compared to unusually high control values.

A correlation analysis of all the groups in the present study indicated that the percentage of females with patent vaginas was highly correlated with the weight of the females at 30 days ($r = .65$; $P < .0001$) but not with the age at breeding the percent pregnant or F1 litter size. Three of the four compounds (benomyl, 6-aminonicotinamide day 11, and cytosine arabinoside) that delayed vaginal opening (Table III) were also smaller than the concurrent control group. Toxaphene delayed vaginal opening without significantly reducing body weight on day 30 but no other reproductive parameters were significantly affected.

DISCUSSION

The present study was an extended evaluation of the mice used by Chernoff and Kavlock [3,4] in their proposed teratology screen. Our results indicate that none of the treatments with normal litter sizes on days 1 and 3 resulted in any mortality up to 250 days of age, indicating that their test would not miss treatments that cause mortality. However, the prolonged assessment through breeding provides a more accurate picture of a chemical's lethal potential. Treatment-induced mortality continued well past the neonatal period in four cases (Table V—nitrofen, benomyl, vitamin A, and cytosine arabinoside), and neonatal viability drastically underestimates the teratogenic potential of cytosine arabinoside.

While neonatal viability provides a useful index for the prioritization of chemicals for subsequent teratologic testing, the use of neonatal growth is a less useful independent parameter to rank chemicals on. Most of those chemicals that did not reduce survivorship but reduced neonatal weight caused only transient growth effects and had been administered at maternally toxic dosage levels. The weight at days 22 or 30 was a better predictor of a prolonged developmental alteration in growth. In addition, one attempt to replicate such an effect on neonatal weight with oral congo red treatment was not successful. The lack of correlation of these early neonatal weights with subsequent postnatal growth is not surprising; some transient growth effects may be due to maternal toxicity rather than being specific fetal effects, and even direct effects on fetal growth may be reversible because many of the hormones that regulate postnatal growth, including growth hormone, thyroxin, cortisol, and androgens, do not play a pivotal role in fetal growth [10].

With all of the variability from block to block between the control groups in the present study one wonders about the ability to replicate treatment effects. Therefore, the success with which we were able to replicate previously published effects of one of the treatments is worthy of note. The postnatal effects of the IP administration of 6-aminonicotinamide at 10 mg/kg body weight on day 15 of gestation (block 9 of the present study) have been described [11]. Chahoud et al [11] observed effects on growth and viability similar to those seen in the present study. They reported that treatment resulted in a 32% reduction in body weight on day 2 (our value is 30% on day 3), a 30% increase in mortality by day 3 (our value is 29%), with no subsequent reduction in viability up to day 8 (we observed a 98% survival rate up to day 30), and the body weights recovered to 99% of control values by day 22 (our mice recovered, but not completely, to 87% of control on day 22). To date the unusual morphological alterations caused by nitrofen, cytosine arabinoside, and congo red have also been replicated.

It is of particular interest that one of the prenatal treatments that did not reduce neonatal survival or produce obvious morphological abnormalities (Table I) produced alterations of reproductive development (congo red, oral). This effect was not detected in the preceeding CKA study [4] and would also be missed in standard teratology bioassay examining fetal mice. Oral administration of the diazo dye congo red during days 8–12 of gestation or IP injection on day 8 was teratogenic to the reproductive system of the male. The testes are normally descended and develop normally until puberty after which they are hypospermatogenic and the Sertoli cells become vacuolated. Subsequent studies in our laboratory have replicated this effect and demonstrated that prenatal exposure to congo red reduces the gametogenic potential of the male but has little effect on testosterone production or the sex accessory glands [8]. Female mice are similarly affected by prenatal congo red treatment, being subfertile [12].

We also found that in addition to these reproductive alterations, other abnormalities develop throughout life as a result of prenatal exposure to chemicals, such as nitrofen and cytosine arabinoside, that cannot be predicted from a careful examination of the newborn pup. Additional studies on the teratogenic potential of nitrofen in the mouse demonstrate that the effect seen in Figure 4 is not due to a direct effect on the eyes but occurs as a consequence of an alteration of Harderian gland development in the orbit [13–15]. This effect is not detectable until after eye-opening because this gland remains small until 2 weeks of age. The effect of cytosine arabinoside on the lower incisors seen in Figure 5 is also undetectable in fetal and neonatal mice unless histological techniques are used. The CNS effects of cytosine arabinoside were anticipated [16] but the inhibition of lower incisor eruption has not been previously reported. Paget and Thorpe [17] found that sulphamoprine, when given in the diet to pregnant rats and mice, also produced abnormalities of incisor eruption in the weanling offspring. They reported that this abnormality could not be detected in the fetal animals even though the fetuses were carefully examined. These results indicate that serious developmental defects, like those seen in the present study, can escape detection because systems that differentiate prenatally but do not function until later in postnatal life cannot be comprehensively evaluated in immature animals.

We feel that the results of this study provide preliminary information on the postnatal teratogenicity of chemicals and can be used along with the CKA to prioritize chemicals for additional study. Our laboratory is conducting additional studies in three species on reproductive and renal effects of nitrofen, studies have been completed using congo red, and other studies are planned using other diazo dyes. In our

subsequent studies the dosing period has been expanded to include late gestation as well as the period of major organogenesis. This change was made because it has been suggested that late gestational exposure can cause functional deficits in the absence of obvious teratologies [18]. For example, reproductive differentiation occurs at this time and during early postnatal life. The sensitivity of the reproductive system to perturbation during early postnatal life is seen in block 19; the estrogenic pesticide chlordecone altered reproductive performance when the female pups were dosed on postnatal day 2, during a sensitive period of sex differentiation of the brain, but gestational exposure at a maternally toxic dosage level on days 8–12 or 14–18 was without effect. In other studies using hamsters, female pups exposed to chlordecone or estrogen after birth abnormally develop male-like behaviors [19] owing to an alteration of sex differentiation of the brain. Unlike the short-term gestation rodents used for most teratology studies, sexual differentiation occurs at the end of the first trimester in humans, and treatment of animals during these critical periods is necessary to assess adequately the teratogenic potential of a chemical.

In summary, the present study was an extended assessment of the animals from the CKA study. Our objective was to evaluate the validity of the use of neonatal viability and weight as the sole parameters to prioritize chemicals for extended teratologic testing. The CKA did not produce false negatives when dams were dosed on days 8–12 of gestation as recommended as all known teratogens reduced neonatal viability and/or weight. However, consideration should be given to extending the observation period beyond day 3 at least through weaning even when compounds are administered on days 8–12 of gestation. Although no treatment caused mortality after day 3 without reducing neonatal viability, some treatments continued to reduce viability and the prolonged observation of the pups more accurately reflects the teratogenic potential of these compounds. In addition, a prolonged observation of the pups provides a much more accurate picture of a compound's potential to adversely affect growth. Pup weight on day 1 and viability may have been influenced by maternal toxicity (Figs. 2, 3) but weights at 22 and 30 days were not. Furthermore, a chemical that causes persistent weight effect should have a higher priority for additional study than one that produces a transient effect. Based on results in the present study using cytosine arabinoside, it would be advisable to observe pups at least through puberty if dosing occurs beyond days 8–12 of gestation. In addition, the effect of congo red on the testis can be detected at this time. An additional recommendation for use of the CKA, indicated by the data analysis presented in the present study, relates to use of the minimally toxic dose (MTD). The potentially confounding effect of reduced maternal weight on neonatal parameters used in the CKA can be avoided by using a dose below that which causes a 20% reduction in maternal weight change during treatment (Figs. 2, 3).

Based on results in the present study using cytosine arabinoside, it would be advisable to observe pups at least through puberty if dosing occurs beyond days 8–12 of gestation. In addition, the effect of congo red on the testis can be detected at this time.

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